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Antibiotic Combinations: Should They Be Tested?

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INTRODUCTION

For a variety of reasons, the use of antibiotic combinations is a common practice in clinical medicine, particularly in the treatment of seriously ill patients. For the most part, such use has been empirical, occasionally based on general principles derived from well-defined bacterial traits or predictable antibiotic activities. Only infrequently has it been necessary to request the assistance of the clinical microbiology laboratory to confirm specifically any beneficial effects of such combinations.

Recent developments, however, now justify a re-examination of the role of the laboratory in testing antibiotic combinations. First, the dissemination of antibiotic resistance determinants which also confer resistance to synergism has made it necessary to reconsider the predictability of certain antibiotic interactions, such as penicillin-gentamicin synergism against enterococci. In addition, there has been increasing awareness of the fact that some antibiotic combinations may be actually antagonistic. This appreciation of undesirable antibiotic interactions has arisen in parallel with a more sophisticated understanding of bacterial resistance mechanisms such as inducibility (derepression) of beta-lactamase production upon exposure to beta-lactam antibiotics. Requests for testing combinations could, therefore, be directed at excluding antagonistic effects between two antimicrobial agents. Finally, growing concerns about hospital costs in general and antibiotic expenditures in particular have provided some motivation to justify the superiority of antibiotic combinations in comparison with monotherapy. This is particularly true when combinations are associated with greater costs of antibiotics or management of drug-related toxicities or both.

The purpose of this paper is to review the role of antibiotic combinations from the point of view of actual clinical

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practice and, in doing so, to examine the circumstances under which the clinical microbiology laboratory might be called upon to render assistance in the evaluation of such combinations.

ANTIMICROBIAL COMBINATIONS: CLINICAL RATIONALE

Any attempt to justify in vitro testing of antimicrobial combinations must begin with an understanding of why such combinations are used. This area has been the subject of extensive examination and of recent reviews (2, 53, 102).

Extension of Antimicrobial Spectrum

One of the most commonly encountered reasons for the use of antibiotic combinations is the desire to provide a broad spectrum of activity during empirical therapy of severely ill patients or when polymicrobial infection is recognized or suspected. The best known example of the former situation involves empirical therapy of the febrile neutropenic patient with suspected gram-negative bacillary sepsis. Here, a delay in the initiation of appropriate antibiotic treatment is likely to be associated with a poor outcome (19, 190). As discussed later, in such circumstances, the exact choice of drugs will often be guided by attempts to provide synergistic bactericidal activity against the etiologic agent (4). Analogous situations exist in other clinical settings such as acute bacterial endocarditis, septic shock, or neonatal meningitis when the "drugs of choice" against likely pathogens are administered with great urgency in the hope of reversing acutely life-threatening infection. To a significant extent, the availability of newer extremely broad-spectrum agents such as imipenem would obviate the need for antibiotic combinations in cases of suspected or proven polymicrobial infection when considered strictly from the point of view of antimicrobial activity (165). However, for reasons of cost and other considerations to be discussed below, combination therapy is still often used in such circumstances.

Minimization of Toxicity

On theoretical grounds, regimens that use two or more drugs in combination, at lower doses than would be used in monotherapy, could circumvent drug toxicity if deleterious effects of individual agents were independent while antimicrobial effects were at least additive. This concept is best illustrated by the use of triple-sulfonamide combinations to avoid the crystalluria observed when relatively insoluble sulfonamides were used in full doses (53). This tactic was effective because the solubility of one agent was not influenced by the presence of the other drugs, while the antimicrobial activities were additive. The development of more soluble sulfonamides and the availability of alternative antimicrobial agents has relegated the triple sulfonamides to a topic of historical curiosity, however. While other attempts to exploit combinations to minimize toxicity have been made (53), in current practice it is rarely possible to titrate doses of individual agents with this purpose in mind. Nevertheless, rationally or not, clinicians not uncommonly use a related maneuver in intentionally underdosing (i.e., aiming for peak serum levels in the low therapeutic range or occasionally lower) potentially toxic drugs such as aminoglycosides while using the "safety net" of a coadministered antibiotic, usually a penicillin or cephalosporin.

Minimization of Resistance

The use of antibiotics in combination to prevent or mini mize the likelihood of emergence of drug-resistant subpopulations is a tactic which has been used for decades in the treatment of tuberculosis (1). Success of this approach is predicated upon independent mechanisms of resistance to the agents. That is, the probability of selecting colonies resistant to both drugs of a combination is approximately equal to the product of the probabilities of resistance to individual agents.

Clinical use of drugs such as rifampin, which is highly active and bactericidal against a number of troublesome pathogens but to which resistance develops easily, as therapeutic agents is generally feasible only in combination regimens. Aside from tuberculosis, one of the most important examples of such use is the treatment of prosthetic valve endocarditis due to coagulase-negative staphylococci with combinations of rifampin plus vancomycin (96). With combination therapy, the otherwise rapid emergence of resistance to rifampin is minimized (8, 48), although not completely prevented. Karchmer et al. (96) noted rifampin-resistant isolates to arise in 2 of 23 episodes of *Staphylococcus epidermidis* prosthetic valve endocarditis treated with this agent in combination with vancomycin or cephalothin or both. Development of rifampin resistance during use of vancomycin-rifampin combinations for *Staphylococcus epidermidis* prosthetic valve endocarditis has been noted by others as well (34). This may reflect emergence of rifampin-resistant clones in deep anatomic foci accessible to this drug but poorly penetrated by vancomycin.

The extent to which use of antibiotic combinations may forestall the emergence of drug-resistant subpopulations during therapy of serious gram-negative bacillary infections has been a matter of controversy. Nevertheless, combination therapy is frequently used with this goal in mind. Support for this approach is provided by some animal studies, examples of which follow. In a model of *Pseudomonas aeruginosa* infection in granulocytopenic mice, addition of ticarcillin to therapy with gentamicin prevented the emergence of aminoglycoside-resistant morphologically variant bacterial colonies (63). In neutropenic rats with *P. aeruginosa* bacteremia, resistance arose to azlocillin, ticarcillin, and amikacin administered as single agents, at frequencies depending upon the size of the bacterial inoculum used. Significantly fewer animals treated with azlocillin-amikacin combinations developed breakthrough bacteremia with resistant strains, and a similar (although not statistically significant) trend was seen with ticarcillin-amikacin combinations (93). Studies by Bamberger et al. (13) examining drug activities against *P. aeruginosa* and *Serratia marcescens* in implanted subcutaneous chambers revealed that combination of ciprofloxacin with azlocillin, ceftizoxime, or amikacin reduced, but did not eliminate, the frequency at which isolates resistant to the fluoroquinolone were detected. Further evidence in support of combination therapy to reduce emergence of resistance is provided by the work of Péchère et al. (145). Short-term therapy with amikacin, ceftriaxone, or pefloxacin as single agents resulted in development of resistance in 25 to 75% of mice treated for peritoneal infection due to *P. aeruginosa*, *Serratia marcescens*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*. For all two-drug combinations of these antimicrobial agents, resistance was detected at lower rates than seen with the individual agents. In other systems, however, it has not been possible to demonstrate any clear-cut advantages of combi-

nation therapy. For example, in a rabbit model of *P. aeruginosa* aortic valve endocarditis, concurrent administration of ceftazidime appeared to offer little protection against the development of amikacin resistance, with subsequent treatment failure, during therapy with this aminoglycoside (16).

Whether or not two-drug combination therapy of gram-negative bacterial infections offers any protection against the development of drug resistance in humans is still not completely settled. Examples can be cited in which combination therapy does appear to offer benefit in this regard. In a prospective randomized trial comparing azlocillin, azlocillin plus tobramycin, and ticarcillin plus tobramycin in the treatment of acute exacerbations of pulmonary disease in patients with cystic fibrosis, drug-resistant isolates emerged during therapy in 53% (8 of 15) of patients receiving azlocillin but in 21% (3 of 14) treated with azlocillin-tobramycin. This difference did not reach statistical significance, however (119). In a prospective comparison of piperacillin monotherapy versus carboxypenicillin-aminoglycoside combination therapy for serious infections, 12 resistant gram-negative isolates emerged among 26 piperacillin-treated patients but in none of 24 receiving the combination (72). The obvious difficulty in this comparison is, of course, that there is no way to determine whether the observed difference was due to the addition of an aminoglycoside or to the choice of penicillin.

On the other hand, other studies fail to support a benefit of combination therapy. In a large (ca. 150 patients in each arm) prospective study comparing aztreonam-vancomycin, aztreonam-amikacin-vancomycin, and moxalactam-ticarcillin, two episodes of superinfection due to resistant gram-negative organisms occurred in the beta-lactam–aminoglycoside group but none occurred in the aztreonam-vancomycin group (94). Further, Chandrasekar et al. (36) reported that, among 14 non-neutropenic patients with *P. aeruginosa* infection treated with combinations of either cefsulodin or ticarcillin plus an aminoglycoside, resistance to the beta-lactam occurred in 7 patients. From a retrospective analysis of 410 episodes of *P. aeruginosa* bacteremia, Bodey et al. (19) concluded that addition of an aminoglycoside to an antipseudomonal beta-lactam yielded no improvement in survival. Although this study did not specifically examine the issue of emergence of resistance during therapy, any net clinical benefit of the combination in this regard must have been quite limited.

While it is clear that neither beta-lactam–aminoglycoside nor beta-lactam–beta-lactam combinations can completely prevent the emergence of resistant gram-negative isolates during therapy (44, 68), many clinicians continue to use the former combinations in the hope that development of resistance will be suppressed or delayed, especially in the treatment of *Pseudomonas* infections.

Synergism

Antibiotic combinations are sometimes used with the specific intent of obtaining a synergistic antimicrobial effect: that is, demonstration of either inhibitory or bactericidal activity which is greater than would be expected merely from the sum of the activities of the individual agents (126).

MECHANISMS OF BACTERICIDAL SYNERGISM

Although antimicrobial agents can interact to produce synergistic inhibitory activity, by far the more interesting interactions are those which result in enhanced rates or absolute magnitudes of bacterial killing by the combination compared with either drug alone. Known mechanisms by which such interactions occur will now be discussed, and appropriate clinical examples will be noted.

Cell Wall-Active Agents Plus Aminoglycosides

Enterococci. Soon after reports of successful combination therapy of enterococcal endocarditis with penicillin plus streptomycin (89, 90), in vitro studies confirmed the synergistic bactericidal interaction of these agents against enterococci (92). Subsequently, the mechanism by which synergism occurs was elucidated by Moellering and Weinberg (130): defined here as $\geq$2-$\log_{10}$ colony-forming units (CFU)/ml reduction in surviving colonies at 24 h in the presence of the combination compared with the most effective single drug alone. In the presence of penicillin, intracellular uptake of [^{14}C]streptomycin by *Streptococcus faecalis* was markedly enhanced in comparison with that observed in the absence of beta-lactam. Other cell wall-active antimicrobial agents such as vancomycin, bacitracin, and cycloserine, which act at different steps in the synthesis of bacterial cell walls and which also produced synergistic killing in combination with streptomycin (131), likewise enhanced uptake of the aminoglycoside. Of interest was the fact that penicillin also enhanced the uptake of streptomycin in a laboratory mutant strain of *Streptococcus faecalis* which was highly resistant (minimal inhibitory concentration [MIC], >2,000 μg/ml) to streptomycin and which was not synergistically killed by this combination. High-level streptomycin resistance in this mutant was shown to be due to insensitivity of the bacterial ribosome to the drug (194). Although ribosomal resistance to streptomycin may account for high-level resistance (MIC, >2,000 μg/ml) to the drug in a small proportion of clinical isolates (51), in the vast majority of cases high-level aminoglycoside resistance and subsequent lack of penicillin-aminoglycoside synergism is due to the presence of aminoglycoside-modifying enzymes. Plasmid-mediated enzymes conferring high-level resistance to streptomycin and kanamycin have been recognized for more than a decade (107); by the late 1970s, approximately 50% of enterococcal blood isolates recovered at the Massachusetts General Hospital were highly resistant to streptomycin or kanamycin or both (28) and thus evaded killing by these agents in combination with penicillin.

In 1979, plasmid-borne high-level resistance to gentamicin was first noted in isolates of *Streptococcus faecalis* from France (84). The nature of the aminoglycoside-modifying enzymes mediating this high-level resistance to gentamicin (and other aminoglycosides) was subsequently defined (39). Since then, enterococcal isolates with high-level gentamicin resistance have been reported from around the world (136) and with increasing frequency in the United States. Such resistant strains have recently accounted for 55% of clinical enterococcal isolates at one institution (193). Of even greater concern is that two isolates of *Streptococcus faecalis*, one from Houston, Tex., and another from Philadelphia, Pa., have been found to possess not only enzymes mediating high-level resistance to aminoglycosides (including gentamicin), but also plasmid-mediated beta-lactamases (133, 135).

Whereas high-level resistance to an aminoglycoside predicts lack of synergism between cell wall-active antibiotics and that aminoglycoside against an enterococcus, absence of high-level resistance does not assure a synergistic interaction. For example, combinations of penicillin with kanamycin, netilmicin, or tobramycin are not synergistic against

most strains of *Streptococcus faecium*, even if MICs of the aminoglycoside are <2,000 μg/ml (127). Resistance to synergism in this case is due to an enzyme characteristic of this species which is capable of acetylating (hence, inactivating) at the 6′ position aminoglycosides such as those just listed with an unblocked amino group in that location (181). However, because amikacin is not a good substrate for this enzyme, combinations of penicillin plus amikacin do exhibit synergism, unless the strain also exhibits high-level kanamycin resistance which reflects the presence of a 3′-phosphotransferase-modifying enzyme as well (181). Strains of *Streptococcus faecalis* possessing the latter enzymatic activity likewise resist penicillin-amikacin synergism despite amikacin MICs as low as 250 μg/ml (29). In fact, against strains producing this enzyme, combinations of penicillin with amikacin are antagonistic. The exact mechanism of such antagonism is still incompletely understood, but it appears to be related to the fact that amikacin is bacteriostatic against enzyme-producing strains but bactericidal at concentrations near the MIC against isolates lacking the enzyme (169). Such enzyme-producing (3′-phosphorylating) strains may be detected by screening for high-level resistance to kanamycin. Finally, a unique clinical isolate of *Streptococcus faecalis* has been described which, although not highly gentamicin resistant (MIC of 8 μg/ml), resists penicillin-gentamicin but not penicillin-tobramycin synergism. This strain demonstrated a specific defect in the uptake of gentamicin, which failed to increase in the presence of penicillin. In the case reported, growth appeared as "small-colony variants," morphologically distinct from typical enterococcal colonies (129).

Streptococci. Combinations of penicillin with streptomycin against viridans streptococci have been studied in vitro (91) and used in the treatment of endocarditis (30) since the beginning of the antibiotic era. Nevertheless, mechanisms of penicillin-aminoglycoside interactions against these organisms, and the clinical significance of such combinations, continue to be areas of active investigation. Yee et al. (189) recently demonstrated in *Streptococcus mitis* enhanced intracellular uptake of streptomycin and tobramycin in the presence of penicillin, with corresponding penicillin-aminoglycoside bactericidal synergism at 24 h of incubation. Against a strain which possessed high-level resistance to streptomycin (MIC, >2,000 μg/ml), penicillin augmented streptomycin uptake without associated synergistic killing. This situation is analogous to what has been described above with enterococci. On the other hand, Miller et al. (122) could demonstrate neither penicillin-induced augmentation of streptomycin uptake nor early synergistic killing of (non-highly resistant) viridans streptococci, in contrast to effects seen against a strain of *Streptococcus faecalis* under the conditions used. Comparison of these two studies highlights the major role that either strain variations or, more likely, methodologic differences play in studies of synergism against viridans group streptococci. It has been our own experience that studies of synergism against these organisms are more difficult to perform reliably and to interpret than are studies examining synergism against enterococci.

In vivo correlation of the enhanced in vitro bactericidal effects of penicillin-aminoglycoside combinations noted against viridans group streptococci has been attempted in several studies with animal models. In a rabbit endocarditis model, combinations of penicillin with streptomycin or gentamicin sterilized cardiac vegetations approximately twice as rapidly as did penicillin alone (157). From this report, however, it is not possible to ascribe definitively any benefit to in vivo "synergism" because an aminoglycoside-only treatment arm was not included and serum concentrations of these drugs nearly reached or actually exceeded MICs of the aminoglycoside components. In a similar experimental model carried out with penicillin-susceptible, -tolerant, and relatively resistant (MIC, 1.0 μg/ml) strains of *Streptococcus sanguis*, Wilson et al. (184) found a benefit of penicillin-streptomycin combinations against the tolerant and relatively resistant isolates. Against the penicillin-susceptible strain, penicillin alone was so active that no advantage of the combination was apparent. Of note is that, at the concentrations selected for study, penicillin-streptomycin synergism could not be demonstrated in vitro against this strain because of excellent efficacy of penicillin alone.

Unfortunately, as is the case for enterococci, strains of viridans group streptococci with high-level aminoglycoside resistance have now been encountered. Among 318 viridans streptococci collected in France between 1978 and 1981, 5.3% demonstrated high-level (MIC, >2,000 μg/ml) resistance to streptomycin or kanamycin or both (86). Such isolates were found among *Streptococcus mitis*, *Streptococcus sanguis* I, *Streptococcus sanguis* II, and *Streptococcus milleri*. Determinants of high-level resistance to the aminoglycoside could be transferred by conjugation into suitable recipient strains in the absence of transfer of detectable extrachromosomal deoxyribonucleic acid (85). High-level streptomycin resistance (MIC, ≥1,000 μg/ml) was recently reported to occur in approximately 2% of viridans streptococcal isolates studied at the Mayo Clinic (56). Farber et al. (57) investigated high-level streptomycin resistance in viridans group streptococci recovered in South Africa and Boston, Mass. In three isolates subjected to intensive study, no evidence of aminoglycoside-modifying enzymes could be found, and the strains could not be "cured" of genetic determinants of such resistance. Rather, studies of ribosomal protein synthesis in a cell-free system demonstrated a relative insensitivity to the inhibitory effects of streptomycin. Strains with high-level streptomycin resistance were not synergistically killed by penicillin-streptomycin combinations, while penicillin plus gentamicin did result in synergy against such isolates. More recently, Farber and Yee (58) have documented the production of streptomycin-adenylylating enzymes by two clinical isolates of *Streptococcus mitis* (streptomycin MICs of 8,000 μg/ml). Both strains were also highly resistant to kanamycin; the ability to phosphorylate the compound was noted in one of these. Genetic determinants mediating production of these enzymes could be neither transferred by conjugation nor cured, and plasmid deoxyribonucleic acid was not detected.

In a rabbit endocarditis model (56), combinations of penicillin plus streptomycin were no more effective than penicillin alone against a highly streptomycin-resistant (MIC, >32,000 μg/ml) strain of *Streptococcus sanguis* II, while penicillin plus gentamicin combination therapy resulted in a 2.5-$\log_{10}$ CFU/g lower mean bacterial titer within vegetations than occurred with penicillin alone. When a strain of *Streptococcus bovis* with a reported streptomycin MIC of 1,000 μg/ml was examined in the same model, no benefit of penicillin-streptomycin therapy was noted. With due regard to the hazards of drawing conclusions from very limited data, it is reasonable to suggest (pending more definitive work in this area) that strains of viridans streptococci with streptomycin MICs of 1,000 to 2,000 μg/ml not be assumed automatically to be susceptible to penicillin-streptomycin synergistic bactericidal effects.

In vitro synergism between penicillin or ampicillin and

streptomycin or gentamicin has also been demonstrated against group B streptococci, which are often slightly more resistant to beta-lactam antibiotics than are other penicillin-susceptible streptococci (11, 45). Combination therapy has resulted in more rapid sterilization of infected cardiac vegetations in a rabbit endocarditis model (11) and in improved survival after septicemic peritonitis in mice (45). High-level resistance to streptomycin and kanamycin (MIC, >2,000 μg/ml) has been detected among clinical isolates of group B streptococci (85). Beta-lactam–aminoglycoside synergism has also been noted among group A streptococci (12). These interactions have not been explored from a mechanistic point of view.

Gram-positive bacilli. Bactericidal synergism between beta-lactam antibiotics and aminoglycosides, as demonstrated by time-kill curve techniques (see below), against *Listeria monocytogenes* has been recognized for more than 15 years (128). Such combinations are often exploited clinically for synergistic effects but, in contrast to enterococci, *Listeria* spp. are often quite susceptible to the aminoglycoside component alone (51).

Similarly, combinations of penicillin with gentamicin often exert a synergistic bactericidal effect against *Corynebacterium* spp. group JK (134). Many isolates of this group are quite susceptible to gentamicin, with MICs of <1.0 μg/ml. However, a significant number of strains are resistant to clinically achievable concentrations of aminoglycosides (these MICs are usually in excess of 128 μg/ml) (134, 167). Penicillin-gentamicin bactericidal synergism has been observed against gentamicin-susceptible strains, but not among the latter group of gentamicin-resistant organisms. Curiously, penicillin resistance (MIC, >128 μg/ml) does not preclude penicillin-gentamicin synergism when clinically achievable concentrations of penicillin are used against aminoglycoside-susceptible strains (134). More recent studies utilizing vancomycin or the cyclic lipopeptide antibiotic designated LY146032 in combination with gentamicin or tobramycin against pathogenic corynebacteria confirmed lack of synergism against aminoglycoside-resistant strains, but demonstrated synergism against aminoglycoside-susceptible isolates inconsistently (167). Demonstration of synergism was dependent upon careful adjustment of antibiotic concentrations to levels just below the MIC; otherwise, synergistic interactions were obscured by the rapid bactericidal activity of the aminoglycoside alone. Mechanisms of synergism against this group of bacteria have not been elucidated.

Staphylococci. That combinations of cell wall-active agents with aminoglycosides may result in enhanced activity against *Staphylococcus aureus* has been long appreciated (180). Such effects have been demonstrated by checkerboard titrations showing both inhibitory and, less commonly, bactericidal synergism (by current definitions; see below) (113, 180). While enhanced activities of such combinations by time-kill curve techniques have been reported, many of these cases do not necessarily reflect true synergism in the sense that this term is used for penicillin-aminoglycoside interactions against enterococci. Instead, published time-kill studies frequently reveal an early bactericidal effect of the aminoglycoside component followed by significant bacterial regrowth by 24 to 48 h of incubation (180). Addition of a beta-lactam serves to prevent such late regrowth. In one study, strains recovered in this late phase after exposure to amikacin alone were found to have a >64-fold increase in minimal bactericidal concentrations (MBCs) of this agent compared with those against the initial isolates (113). Enhanced killing by a combination under these circumstances is probably more appropriately considered to be due to prevention of the emergence of resistance, rather than true synergism.

This in vitro phenomenon has been amply borne out in animal experiments. In a study of *Staphylococcus aureus* endocarditis in rabbits, combinations of nafcillin with gentamicin more rapidly sterilized vegetations compared with nafcillin therapy alone, although this agent alone sterilized vegetations in animals treated for more than 7 days. Vegetations from animals receiving gentamicin alone revealed gentamicin-resistant dwarf colonies (156). In a similar model (124), nafcillin alone or with gentamicin yielded equivalent results on day 2 of therapy, although by days 4 and 6 of therapy an advantage of the combination was evident. Small-colony morphologic variants were isolated from animals receiving gentamicin alone, with a 32-fold increase in gentamicin MICs compared with the initial isolate. In vitro, dwarf colony gentamicin-resistant mutants can be selected at frequencies of approximately 10^{-7} (83).

Studies recently reported by Zenilman et al. (192) provide additional information regarding beta-lactam–aminoglycoside interactions against *Staphylococcus aureus*. Concentrations of oxacillin above the MIC, in combination with subinhibitory concentrations of streptomycin, resulted in marked stimulation of streptomycin uptake and an approximately 1.25-$\log_{10}$ CFU/ml enhancement of killing by the combination (compared with oxacillin alone) at 150 min of incubation. With longer incubation (3 to 4 h), a >2-$\log_{10}$ CFU/ml increase in killing by the combination was seen against a second isolate. At streptomycin concentrations above the MIC (as were commonly used in earlier studies of drug interactions), addition of oxacillin did not augment intracellular [^{3}H]streptomycin uptake and resulted in an additive bactericidal effect only. Thus, when studies with *Staphylococcus aureus* are performed with methods analogous to those used for enterococci (streptomycin at subinhibitory concentrations; beta-lactam at concentrations just exceeding the MIC), then stimulated aminoglycoside uptake and true bactericidal synergism can be demonstrated with this species also. Zenilman et al. hypothesized that release of surface teichoic acids during beta-lactam exposure permits increased intracellular penetration of the aminoglycoside, the access of which is normally hindered by the presence of the negatively charged surface polymers.

Gram-negative bacilli. In 1962, Plotz and Davis (194) examined interactions between penicillin G (at high concentrations) and streptomycin against *Escherichia coli*. Prior penicillin exposure enhanced the bactericidal activity of streptomycin and augmented the uptake of [^{14}C]streptomycin by bacterial cells. These authors proposed that injury to the cell envelope by penicillin resulted in increased penetration by the aminoglycoside. Since that time, numerous studies utilizing various techniques and definitions have provided evidence for beta-lactam–aminoglycoside synergism against gram-negative bacilli (64). Although considerable attention has been given to the study of interactions against the *Enterobacteriaceae* (40, 66, 67, 95, 101), much attention has also been focused on activities of combination regimens against *P. aeruginosa*, no doubt due to difficulties in treating many such infections in seriously ill patients (19).

Although it had been widely held (without proof) that mechanisms of beta-lactam–aminoglycoside synergism against other gram-negative bacilli were similar to those elucidated in *E. coli* (149), other possibilities have been explored. Although, as discussed above, combination regi-

TABLE 1. Synergism of beta-lactams with aminoglycosides against the *Enterobacteriaceae*

Organism and drug combination	Method[a]	% of strains showing synergism	Reference
Klebsiella spp.			
Cephalothin-aminoglycoside	MIC	~80	40
Cefazolin-aminoglycoside	MIC	80	101
Cefazolin-aminoglycoside	MBC	65	101
Cefazolin-aminoglycoside	TKC	80	101
Imipenem-amikacin	MIC	5	73
Ceftazidime-amikacin	MIC	30	73
Cefotaxime-amikacin	TKC	83	67
Enterobacteriaceae			
Ticarcillin-tobramycin	MIC	45	38
Piperacillin-tobramycin	MIC	16	59
Moxalactam-amikacin[b]	MIC	54	95
Cefoperazone-amikacin	MIC	70	95
Carbenicillin-gentamicin	TKC	13	66
Piperacillin-gentamicin	TKC	52	66
Piperacillin-amikacin	TKC	90	66
Cefotaxime-amikacin[c]	TKC	85	67
Piperacillin-amikacin	TKC	81	67
Moxalactam-amikacin	TKC	67	67

[a] MIC, Checkerboard titrations with inhibitory endpoints; MBC, checkerboard titrations with bactericidal endpoints; TKC, time-kill curves.
[b] Organisms moderately resistant to expanded-spectrum cephalosporins.
[c] Organisms multiply resistant.

mens might minimize the emergence of strains resistant to one or the other antibiotic (55), documentation of synergistic effects between beta-lactams and aminoglycosides in the absence of resistance to the individual agents (153) supports the possibility of additional mechanisms of favorable interactions. Hancock et al. (78, 79, 114) demonstrated that exposure of intact organisms to aminoglycosides enhanced permeability of the outer cell membrane to various compounds, including the chromogenic cephalosporin nitrocefin. Further support for a primary role of aminoglycoside-mediated membrane damage arose from electron microscopic studies demonstrating major structural pertubations of the bacterial cell wall, beginning at the outer membrane, in *P. aeruginosa* exposed to gentamicin at or above the MIC (118). Unlike the intracellular uptake of aminoglycosides, which is an energy-dependent process (23), the ultrastructural damage induced in these experiments proceeded even in the presence of metabolic inhibitors (potassium cyanate or sodium azide).

Nevertheless, indirect evidence that aminoglycoside-induced outer membrane permeabilization could not fully account for synergism against *P. aeruginosa* derived from studies showing that, while activities of carbenicillin and gentamicin individually were increased after treatment of test strains with ethylenediaminetetraacetic acid (which increases permeability), synergistic interactions were not affected by this maneuver (162). Finally, direct evidence has now been presented that beta-lactam–aminoglycoside synergism against *P. aeruginosa* can occur by mechanisms directly analogous to those previously documented in *E. coli*, enterococci, and *Staphylococcus aureus*. Miller et al. (123) have demonstrated that both ticarcillin and cefsulodin could enhance the intracellular uptake of [^{3}H]tobramycin by *P. aeruginosa* over a period of a few hours and, within the same time frame, result in bactericidal synergism by time-kill curve methods. While this study provides long-awaited support for a widely held view, confirmation of such a mechanism certainly does not exclude aminoglycoside-mediated outer membrane damage or cooperative suppression of resistance as an important contributing factor to the overall effect of such combinations in clinical situations.

A number of studies have examined potential synergistic activities of beta-lactam–aminoglycoside combinations against the *Enterobacteriaceae*. Several of these are summarized in Table 1. Against *Klebsiella* spp., synergism between cephems and aminoglycosides was noted in 65 to 95% of isolates, depending upon the method and particular combination used. With the newer beta-lactams, results vary even more widely. The frequency at which synergism is documented against the *Enterobacteriaceae* depends upon susceptibilities of organisms to the individual agents, ranging in one study from 70 to 80% for strains susceptible to one or both drugs to 40% when strains were resistant to both components of the combination (67).

Similar data for *P. aeruginosa* are shown in Table 2. It has been long appreciated that low-level aminoglycoside resistance in this species does not preclude beta-lactam–aminoglycoside synergism (104, 176); resistance to the beta-lactam component likewise does not exclude the possibility of synergistic interactions (9, 138). In one study, among strains resistant to each component of a combination, synergism was seen in 13 to 57% of isolates, depending upon the particular combination used (117). The main conclusion that can be drawn from such studies is that it is difficult to predict from strain characteristics and in vitro susceptibility to individual agents whether a particular combination will exhibit synergistic activity against any one clinical isolate at pharmacologically relevant concentrations.

TABLE 2. Synergism of beta-lactams with aminoglycosides against *P. aeruginosa*

Combination	Method[a]	% of strains showing synergism[b]	Reference
Ticarcillin-aminoglycoside	MIC	76	82
Imipenem-amikacin	MIC	45 IMI^s, 10 IMI^r	26
Imipenem-amikacin	MIC	5	73
Ceftazidime-amikacin	MIC	30	73
Azlocillin-amikacin	MIC	55	73
Piperacillin-tobramycin	MIC	23	59
Moxalactam-tobramycin	MIC	66	59
Piperacillin-amikacin	MIC	55 AM^s, 88 AM^r	110
Moxalactam-amikacin	MIC	79 AM^s, 50 AM^r	110
Cefoperazone-tobramycin	MIC	21	125
Carbenicillin-tobramycin	MIC	26	125
Cefotaxime-tobramycin	MIC	63	125
Ticarcillin-tobramycin	MIC	35	38
Ticarcillin-tobramycin	MBC	39	38
Antipseudomonal penicillin-aminoglycoside	MBC	53–78	117
Moxalactam-gentamicin	MBC	33	191
Piperacillin-tobramycin	TKC	45	179
Piperacillin-gentamicin	TKC	15	179
Azlocillin-netilmicin	TKC	71	138
Mezlocillin-netilmicin	TKC	50	138

[a] MIC, Checkerboard titrations with inhibitory endpoints; MBC, checkerboard titrations with bactericidal endpoints; TKC, time-kill curves.
[b] IMI^s, IMI^r, Imipenem susceptible or resistant; AM^s, AM^r, amikacin susceptible or resistant.

It has been difficult to draw firm conclusions relating in vitro synergism against gram-negative bacilli to in vivo efficacy in animal models. There are at least three reasons for this: (i) most papers, including those purporting to document benefits of synergism, study animal models with strains that have shown in vitro synergism, without comparative strains which are not affected synergistically; (ii) in vitro data, as presented, are often not adequate to differentiate true synergism (based on mechanisms discussed in this section) from mutual suppression of resistant subpopulations; and (iii) there is no generally accepted standard definition of "in vivo synergism." For the most part, therefore, benefits of combination therapy in vivo are ascribed based on the statistical significance of comparative data (survival, sterilization of blood or infected sites, colony counts of residual bacteria, etc.). However, even if statistically significant, such differences are not always of a magnitude likely to reflect clinical importance. This is understandable because, even in human clinical trials, superiority of one regimen relative to another by one measure of response (e.g., more rapid clearance of bacteremia) is not necessarily predictive of superior clinical efficacy (105).

The predictive value of in vitro tests for in vivo outcome was examined by Andriole (6) in a rat model of *Klebsiella* septicemia. Combinations of carbenicillin or cephalothin with tobramycin or gentamicin were examined in vivo against three isolates, one of which was synergistically killed by all combinations, another of which was resistant to synergism by any combination, and a third of which was synergistically killed by some but not all combinations. By using mortality rate comparisons between combinations and individual agents as indicators of in vivo synergism, the results of in vitro testing predicted experimental outcome for 11 of 12 organism-drug regimen combinations. Other examples of animal models which suggest a benefit for synergistic combinations can be cited (5, 7, 27, 33, 61, 112). However, not all studies clearly indicate advantages of synergistic regimens. Norden and Shaffer (140) found no benefit of combination therapy for *P. aeruginosa* osteomyelitis in comparison with single-drug therapy despite documentation of in vitro synergism by two methods.

Enzyme Inhibitors

Beta-lactamase inhibitors. The elaboration of beta-lactamases is a major mechanism of resistance to beta-lactam antibiotics in both gram-positive and gram-negative bacteria. For the most part, attempts to overcome this mechanism of resistance have focused on the development of new beta-lactam antibiotics with greater resistance to beta-lactamase-mediated hydrolysis. Another approach has been to combine a hydrolyzable (but otherwise intrinsically active) beta-lactam with another beta-lactam of higher affinity for the enzyme, the latter acting as an inhibitor of the enzyme. Synergism by combinations such as ampicillin-cloxacillin and hetacillin-dicloxacillin against gram-negative bacilli has been demonstrated in vitro (21, 24), and such combinations have been successfully used in the treatment of urinary tract infections caused by beta-lactamase-producing organisms resistant to the aminopenicillin (155). In such cases, isoxazolyl penicillins serve as competitive enzyme substrates (because they are slowly hydrolyzed) (25). However, high concentrations of these drugs required for activity render them essentially useless for infections beyond the urinary tract. Much more potent inhibitors of various beta-lactamases of both gram-positive and -negative organisms are two naturally occurring substances, clavulanic acid and sulbactam, which themselves possess only weak intrinsic antimicrobial activity (25, 139, 187). These compounds act as suicide inactivators of the bacterial enzymes, meaning that both drug and enzyme are destroyed subsequent to their interaction (25). Combination of potassium clavulanate with amoxicillin extends the spectrum of the latter to include many beta-lactamase-producing strains of *Staphylococcus aureus*, *Haemophilus influenzae*, *Bacteroides fragilis*, *K. pneumoniae*, and some other members of the *Enterobacteriaceae* (70, 174). Combined with ticarcillin, the inhibitor increased the proportion of the *Enterobacteriaceae* susceptible to the former from 72 to 91% (14). The combination also demonstrates enhanced activity against *B. fragilis* and possesses moderate activity against methicillin-susceptible *Staphylococcus aureus* (14). Sulbactam extends the spectrum of ampicillin in much the same way as clavulanic acid does, with minor differences in drug potency and activity against specific enzymes (2, 70). Ampicillin and sulbactam moieties have been linked chemically (sultamicillin) in the form of an orally well-absorbed, mutual prodrug ester compound hydrolyzed in vivo to yield the parent drugs in higher serum concentrations than would be attainable with either alone at equivalent dosage (50). Unfortunately, neither potassium clavulanate nor sulbactam provides useful inhibitory activity against the Richmond and Sykes class I inducible, chromosomally mediated enzymes which confer resistance to beta-lactams in some species of gram-negative bacteria, including *P. aeruginosa* and *Enterobacter cloacae* (2).

Bactericidal synergism is occasionally seen with combinations of hydrolyzable beta-lactams plus agents affecting beta-lactamase synthesis. Combinations of chloramphenicol at low concentration with beta-lactams were found to be synergistic against 20% of gram-negative bacilli resistant to the latter by virtue of beta-lactamase production. Failure of chloramphenicol to inactivate enzyme directly suggested that the synergistic effect was due to inhibition of enzyme production (121). However, such effects are very concentration dependent; higher concentrations of bacteriostatic agents such as chloramphenicol may antagonize bactericidal activities of beta-lactams. More recently, Sanders et al. (159) have shown that clindamycin has the potential to inhibit derepression of the inducible chromosomal beta-lactamases found in *P. aeruginosa* and *Enterobacter cloacae*. Constitutive production of enzyme by a fully derepressed mutant strain of *Enterobacter cloacae* was unaffected by addition of clindamycin. Combination of clindamycin (as an inhibitor of beta-lactamase derepression) with cefamandole enhanced the bactericidal activity of the latter against *Enterobacter cloacae* both in vitro and in vivo.

Aminoglycoside-modifying enzyme inhibitors. Compounds have been discovered which inhibit the activity of aminoglycoside-modifying enzymes. One such compound is 7-hydroxytropolone, an inhibitor of the 2''-O-adenylylating enzyme found in some gram-negative bacteria (3). This compound potentiates the activity of gentamicin against enzyme-producing strains of *E. coli*. Agents of this class are not available for clinical use at the present time.

Sequential Blockade of Metabolic Pathways

Trimethoprim-sulfamethoxazole. The combination of trimethoprim and sulfamethoxazole exemplifies synergism resulting from blockade of sequential steps of one critical metabolic pathway. Inhibition of dihydropteroate synthetase and dihydrofolate reductase, steps in folic acid synthesis,

results in bactericidal synergism against many isolates only inhibited by the individual components (37, 81). Antimicrobial activity of the combination extends to cover a wide range of gram-positive and -negative organisms, including many multiple resistant nosocomial pathogens (160, 161). Recently, this drug has been used extensively in the treatment of *Pneumocystis carinii* infection, although the frequency of adverse reactions to the combination is high among patients with the acquired immunodeficiency syndrome (88).

Other agents. Blockade of multiple steps along a metabolic pathway has also been invoked to explain the bactericidal synergism seen when beta-lactams (which inhibit late steps in peptidoglycan synthesis) are combined with drugs such as fosfomycin (an agent acting at an early step in cell wall synthesis) (53). However, more recent data suggest that fosfomycin may also indirectly influence the activity of beta-lactams by affecting the synthesis of penicillin-binding (target) proteins (PBPs) (172). Other combinations designed to block sequential early steps in cell wall synthesis have been examined (53, 186). One such example is the combination of fludalanine, an inactivator of alanine racemase, with cycloserine (or the prodrug, pentizidone), an inhibitor of D-alanyl-D-alanine synthetase (186).

Double Beta-Lactam Interactions

Recognition that beta-lactam antibiotics have preferential affinities for specific targets (PBPs) on the bacterial inner cell membrane (170) has led to the concept that combinations of agents with high affinities for complementary PBPs may produce synergistic effects (137). This is best illustrated by combinations of amdinocillin, an agent with specific activity for PBP 2 of *E. coli*, with agents such as aztreonam which bind preferentially to PBP 3. At concentrations of the latter which are only bacteriostatic, addition of amdinocillin results in rapid cell lysis (74). Further support for this hypothesis derives from work with mutant strains possessing thermosensitive PBP 2 or 3 and compounds with specific affinities for PBPs complementary to the nonfunctioning targets.

Other combinations of beta-lactam antibiotics have been cited as showing synergism against a variety of gram-negative bacilli (75). In most cases, mechanisms of such interactions have not been studied. Presumably, in such situations, synergism is due either to binding to complementary PBPs or to one agent serving as a beta-lactamase inhibitor, protecting the second intrinsically more active drug from hydrolysis. The latter explanation is the more likely for combinations of penicillins with expanded-spectrum cephalosporins.

Beta-lactam combinations may also exhibit antagonistic effects (2, 75). Unlike beta-lactam–aminoglycoside combinations (with which antagonism has been reported but appears to be distinctly uncommon), antagonism of one beta-lactam by another is not rare (59, 95, 109, 110, 138, 179). For example, Neu and Fu (138) detected antagonism between azlocillin and cefazolin against 16% of gram-negative bacilli tested. Frequencies of reported antagonism vary widely depending upon methods used, antibiotics combined, and species examined (132). While other mechanisms of antagonism are theoretically possible, the most common reason for antagonism appears to be derepression of beta-lactamase production in the presence of a potent inducer (with poor intrinsic antimicrobial activity), with subsequent inactivation of the more active member of the combination (2, 75). An example of this phenomenon is the antagonism of the activity of cefamandole against *Enterobacter cloacae* in the presence of cefoxitin, which occurs both in vitro and in vivo (159). Derepression of chromosomally mediated beta-lactamases has recently been shown to be a more complex process than initially appreciated, involving two or more stages of genetic control and possibly synthesis of multiple enzyme forms (62).

METHODS FOR ASSESSING DRUG INTERACTIONS

In reviewing possible mechanisms of synergism in the preceding section, detailed discussion of methods used to document in vitro interactions has been purposely avoided. Despite the fact that each of the techniques which have been described is intended to reveal synergistic or antagonistic interactions which might be clinically relevant, the precise methods, endpoints, and interpretative criteria used have varied widely and have been the subject of considerable debate (77). In addition, all of these methods have been criticized for the fixed drug concentrations and sampling times used, and alternative techniques to permit dynamic assessment of interactions have been presented (22, 46, 98). A detailed description and analysis of these methods is beyond the scope of this paper and can be found elsewhere (108). This section will, instead, summarize methods which have been used most commonly in studies relevant to clinical practice, indicating potential advantages and limitations of each method. These limitations are discussed not to discourage use of such tests but rather to point out areas of potential difficulty in interpretation of published reports and to highlight aspects requiring special attention when synergy testing is contemplated.

Checkerboard Titrations

The most frequently used method to study antimicrobial interactions involves formation of a checkerboard array representing all possible combinations of two antibiotics serially diluted within a desired range (108). Most commonly, the test is designed to assess inhibitory effects only, in which case it can be carried out with either broth or agar media. Serial twofold dilutions of antibiotics have been generally used; however, some authors have recommended smaller concentration intervals to permit greater precision (77, 87).

With this technique, synergism is usually defined as occurring when combinations of two drugs, each at one-fourth MIC or lower, inhibit growth. This is often expressed in terms of a fractional inhibitory concentration (FIC) index equal to the sum of FICs for each drug, defined as: FIC = MIC of the drug in combination divided by MIC of the drug used alone (17, 49). By this definition, synergism is said to occur when the FIC index is ≤ 0.5. Beyond definitions of synergism, there is little unanimity of opinion regarding classification of other interactions. For example, while the most commonly used criterion for antagonism has been a FIC index of ≥ 2, criteria ranging from a FIC index of >1 to one of >4 have been used (77, 108). Another American Society for Microbiology journal, *Antimicrobial Agents and Chemotherapy*, has adopted as a definition of antagonism the latter criterion (FIC index of >4).

The checkerboard test can be extended to investigate bactericidal drug interactions by sampling from tubes or wells onto antibiotic-free media to determine MBCs of each drug alone and in combination. Results can then be ex-

pressed in terms of fractional bactericidal concentration index, analogous to the FIC index described above. However, using the checkerboard test to assess bactericidal activity not only substantially increases the work involved, but also introduces additional difficulties. For example, in performing MIC broth tests, inocula of ca. 10^5 CFU/ml are preferred (108). On the other hand, use of inocula this low substantially reduces the accuracy of MBC testing when standard 10-μl aliquots are sampled (144). Solutions to this dilemma (when both MIC and MBC data are desired) include repetition of the test at two inocula or use of larger sample volumes, in which case problems of antibiotic carryover must be dealt with.

Time-Kill Techniques

Time-kill techniques involve repeated sampling of tubes or flasks containing the individual drugs and their combinations to determine colony counts of surviving bacteria over time (108). As with any test of antibiotic activity over time, matters of inoculum size and growth phase, medium composition, and possible in vitro drug inactivation must be considered (65, 120, 147, 195). The last of these factors is particularly important when broad-spectrum penicillins are combined with aminoglycosides. In this situation, significant inactivation can occur in vitro (147, 177) as well as in vivo (41, 151).

Although offering an advantage over checkerboard tests in that both rate and extent of killing can be assessed, the time-kill procedure is often more labor intensive. Therefore, for any organism studied, only a limited number of antibiotic concentrations (alone and in combination) can be examined. Proper execution of a synergism study by this technique may require one or more preliminary experiments to establish the concentrations of individual agents most likely to permit detection of any synergistic interaction.

Accurate assessment of bactericidal activity also requires that measures be taken to avoid carry-over of antimicrobial agents during sampling of broth media onto drug-free plates used for determination of colony counts. Obviously, inadvertent transfer of antibiotics at inhibitory concentrations may lead to overestimation of bactericidal effects. Some drugs can be chemically or enzymatically inactivated. For example, many penicillins can be effectively removed either by addition of penicillinase directly to sample aliquots or by incorporation of enzyme into the counting plates. For agents which cannot be inactivated without destruction of viable bacteria, possible approaches include washing the sample aliquots over membrane filters prior to plating or demonstrating that residual antibiotic is diluted to insignificant (i.e., subinhibitory) concentrations upon transfer to counting plates.

Time-kill methodologies for the study of combinations of cell wall-active agents with aminoglycosides against enterococci have been well established (108). In this model system, subinhibitory concentrations of the aminoglycoside are selected. Preferably, such concentrations cause little or no deviation from growth seen in antibiotic-free broth (71, 126). Usually, concentrations of the cell wall-active drug are selected which cause growth inhibition or slight killing to maximize the likelihood of detecting synergism. Against enterococci, beta-lactam antibiotics often demonstrate a paradoxical bactericidal effect, showing maximal killing at low concentrations with progressively reduced bactericidal rates as drug concentration is increased (47). Such effects must be taken into account when trying to establish the existence of synergism. Often, several concentrations of the cell wall-active agent must be studied. Typically, samples are withdrawn for colony counts at 4 and 24 h of incubation.

Unfortunately, there does not currently exist any generally accepted standard method for performing time-kill studies on gram-negative bacilli (108, 126). Few studies use drug concentrations analogous to those used in the enterococcal model just described. In some cases, growth inhibition or moderate killing by each agent at the concentrations selected makes it difficult or impossible to distinguish synergism from an "additive" effect (17). In other situations, one or both drugs cause significant early killing followed by subsequent regrowth, occasionally to levels reaching those in antibiotic-free control flasks. Under these conditions, it is usually not possible to distinguish true synergism from suppression by one drug of emergence of resistance to the second agent.

Definitions of synergism in time-kill methods generally require ≥100-fold killing by the combination compared with the most active single agent at a designated sampling time. For enterococci and listeria, this is generally taken to be at 24 h of incubation (52). However, with agents used against gram-negative bacilli, incubation periods of that duration may result in almost complete inactivation of several beta-lactam antibiotics because of either enzymatic hydrolysis or chemical deterioration. As a result, shorter incubation periods before sampling have been recommended (66). Antagonism is often defined as a 100-fold reduction in killing by the combination compared with that seen with the most active drug alone (108). However, in some circumstances, adverse influence of one antibiotic on the activity of another of smaller (<2-$\log_{10}$ CFU/ml) magnitudes occurs in a highly reproducible manner, convincingly suggesting antagonistic interactions (29, 169).

Diffusion Tests

Disk diffusion tests easily assess net antimicrobial activities of combinations for which commercially prepared disks containing both drugs are available (trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, ticarcillin-clavulanate). For other combinations, double-disk diffusion techniques have been devised to qualitatively determine drug interactions (108). Antibiotic-impregnated disks are approximated on the surface of an agar plate streaked with the test organism in a manner similar to that for Bauer-Kirby testing. Evidence of synergism is provided by elongation of the zones of inhibition of one or both drugs in the direction of the adjacent disk. Antagonism appears as truncation of the inhibitory zone(s) in the direction of the adjacent disk. Techniques have been developed to transform such tests of bacteriostatic interactions into qualitative assays of bactericidal activity (20, 31, 32).

Correlation of Results by Various Methods

It should be obvious that each of the various tests described potentially measures quite distinct effects of antibiotic interactions against bacteria. Furthermore, for any one method, lack of standardized methodology or interpretative criteria contributes to the broad range of results presented in the literature (Tables 1 and 2). As a consequence, correlation of results by different methods is often disappointing. For example, assessment of penicillin-aminoglycoside interactions against enterococci by MIC checkerboard techniques correlate poorly (<5% agreement) with results of time-kill studies when currently accepted criteria for synergism are

used (154). Similarly, comparison of beta-lactam–aminoglycoside interactions against *P. aeruginosa* by checkerboard MIC testing and 6- or 24-h time-kill curves results in concordance rates of <50% between any pair of methods (35, 36). On the other hand, several studies examining small numbers of strains have suggested rough correlations between results obtained by different methods (110, 191). Klastersky et al. (101) found agreement between time-kill and MBC checkerboard assessment of cefazolin-amikacin interactions for 60% (13 of 20) of *Klebsiella* isolates. Norden et al. (141) illustrated discrepancies when various methods and criteria were applied in examining cephalothin-gentamicin interactions against *Klebsiella*, but, in general, synergism by MIC checkerboard testing did predict synergism by time-kill methods (although the reverse was not true). In a study which examined several hundred gram-negative bacilli, synergism by MIC checkerboard techniques predicted synergism by MBC criteria in approximately 80% of strains (4).

RATIONALE FOR CLINICAL TESTING OF COMBINATIONS

Several assumptions underlie decisions to perform in vitro tests of antimicrobial interactions in specific clinical settings. Foremost is the concept that, for whatever reason, the unnecessary use of more than one drug is undesirable. This approach is justified from the point of view of adverse reactions alone. Common adverse reactions including rashes from aminopenicillins (164) or nephrotoxicity from aminoglycosides (53) occur in 8 to 10% of patients receiving therapeutic courses of these drugs. Exposure to multiple drugs clearly places the patient at substantial risk of suffering a drug reaction or drug-induced toxicity. Also of increasing concern is the cost of antimicrobial therapy. Financial pressures have resulted in closer scrutiny not only of microbiology laboratories (178), but also of hospital formularies. Based on current prices at our institution, full-dose therapy with the least expensive extended-spectrum acylaminopenicillin (including administration charges) costs approximately $40 per day. Addition of standard intravenous doses of an aminoglycoside almost doubles that cost when charges for once-weekly peak and trough serum levels are included.

Also, there is ample evidence to suggest that excessive antibiotic use exerts pressure for the selection and maintenance of resistant bacterial isolates in the hospital environment (166). Limitation of unnecessary use of multiple antibiotics most likely would minimize such selective pressures. However, this is counterbalanced by the possibility that in the individual patient combination therapy might delay or suppress the emergence of resistant strains, as discussed above.

To justify the effort and expense of testing antibiotic combinations, there must be reason to believe that synergism would enhance clinical efficacy, as in the case of penicillin-aminoglycoside combinations in the therapy of enterococcal endocarditis (158), or that in vitro antagonism would predict clinical failure. Many situations in which in vitro antagonism has been associated with clinical failure are currently predictable based on general principles (for example, combination of penicillins with tetracyclines in the treatment of meningitis [111]), and do not justify testing. In other cases, concern about potential antagonism (e.g., with double beta-lactam therapy of gram-negative rod infections [132]) might lead to requests for in vitro assessment. Unfortunately, in most of these situations, while demonstration of florid antagonism would discourage the use of a particular combination, failure to detect an adverse interaction cannot at this time be taken as definitive evidence to exclude the possibility of antagonism in vivo. Rarely, multiply resistant nosocomial pathogens might be encountered against which available single agents are ineffective. In some instances, combinations may provide activity against organisms resistant to each component of the regimen (9).

TESTING IN SPECIFIC CLINICAL SITUATIONS

Enterococci

There is ample evidence that treatment of serious enterococcal infections such as endocarditis requires bactericidal therapy which, given currently available drugs, generally means combinations of penicillin, ampicillin, or vancomycin with an aminoglycoside (158). Although time-kill techniques sometimes demonstrate synergism between aminoglycosides and other cell wall-active agents, clinical use of such combinations is ineffective or impractical. For example, in vitro synergism between nafcillin and gentamicin is negated by addition to test media of human serum, to which the former is highly bound, and such combinations are ineffective in animal models (65).

Resistance to synergism between penicillins and gentamicin in strains which do not possess high-level resistance to gentamicin has been documented, but is probably very rare (129). Therefore, for practical purposes, screening for high-level resistance to streptomycin or gentamicin is preferable to detailed time-kill studies except in unusual circumstances such as in assessing causes of treatment failure. Amikacin represents an exception to this rule in that resistance to synergism is usually not manifest by extraordinarily high MICs; however, high-level (MIC, >2,000 μg/ml) resistance to kanamycin is predictive of the presence of a 3′-APH (aminoglycoside-phosphorylating) enzyme which renders amikacin inactive in terms of synergism (29). Also, the presence of 6′-AAC (acetylating) enzymes in most isolates of *Streptococcus faecium* renders tobramycin, netilmicin, and other aminoglycosides susceptible to modification at that position unable to participate synergistically with penicillin, even in the absence of high-level resistance (127, 181). For streptomycin, failure of organisms to grow on plates containing 2,000 μg of the drug per ml results in a high probability that penicillin-streptomycin combinations will be synergistic (28, 168). Appropriate concentrations of gentamicin for use in high-level resistance screening have not yet been formally established. Pending further data, use of 500-μg/ml concentrations for screening appears prudent. Strains which grow at this drug level could be retested at higher concentrations or subjected to formal time-kill curve study. Lack of growth at 500 μg/ml should provide reasonable assurance of synergistic potential.

Staphylococci

Despite extensive debate, the utility of adding aminoglycosides to antistaphylococcal penicillins in the treatment of serious *Staphylococcus aureus* infections remains controversial (53). It has long been appreciated that some strains appear to be "tolerant" of the bactericidal activities of cell wall-active agents; i.e., either the MBC >> MIC or the rate of bacterial killing is slow (80). Two factors have hampered experimental investigation of the significance of this phenomenon: (i) demonstration of phenotypic (as opposed to genotypic) tolerance is highly method dependent, and (ii)

suitable standard reference strains have not been available. Recently described, tolerant laboratory mutants of *Staphylococcus aureus* may eventually serve in the latter role (171).

Clinical studies have shown variable benefits of combination therapy. In a multicenter prospective study in which patients with *Staphylococcus aureus* endocarditis were randomized to receive nafcillin alone or in combination with gentamicin, addition of gentamicin resulted in somewhat more rapid clearing of bacteremia (2.8 versus 4.1 days, in non-drug addict patients) at the cost of more renal dysfunction. Ultimately, there were no significant differences in serious morbidity or mortality (105). Combinations of vancomycin with rifampin have been used in the treatment of staphylococcal infections with reportedly good effect (60). However, in a double-blind, placebo-controlled study in which staphylococcal infections were treated with oxacillin alone or in combination with rifampin, no significant advantage of combination therapy was noted despite higher serum bactericidal titers (SBTs) 6 and 11 h following dosing (175). Studies measuring SBTs on "spiked" samples of serum have shown that rifampin can actually decrease both SBTs and serum bactericidal rates against *Staphylococcus aureus* when added to nafcillin (76). The activity of vancomycin was diminished by rifampin as measured by the former method but not the latter. However, addition of either drug to rifampin prevented the emergence of rifampin-resistant isolates, seen when rifampin was used alone. Much of what has been said about antibiotic combinations against *Staphylococcus aureus* pertains to coagulase-negative staphylococci as well. Infections due to these organisms are often nosocomially related or foreign-body associated or both. As a result, multiply resistant strains are not uncommon (116). Combination antibiotic therapy is used frequently in these settings, generally to maximize the utility of rifampin, which is highly active against most isolates, but to which resistance arises rapidly (8).

In view of the fact that results of testing such combinations by various methods do not always reveal concordant interactions, it is not surprising that in vitro data do not always predict in vivo efficacy (15). Interactions between various agents often used in combination for the treatment of staphylococcal infections may be quite complex, including elements of synergism, antagonism, and suppression of resistant subpopulations, and may be method and concentration dependent. Therefore, we believe that there is little justification for routinely performing studies of such combinations in the clinical laboratory. To the extent that high peak or trough SBTs reassure the clinician (188), these may be determined during empiric combination therapy or before and after addition of a second agent to first-line antistaphylococcal therapy.

Viridans Streptococci

Increasing interest on the part of physicians to minimize duration of hospitalization during treatment of bacterial endocarditis by utilizing 2-week penicillin-streptomycin regimens (183) and recognition that relatively penicillin-resistant viridans streptococci can, on occasion, produce serious infection (142) has led to a more critical focus on the use of penicillin-aminoglycoside combinations against this group of organisms. Wilson (182) has recently reviewed considerations in the choice of antibiotic regimens for treatment of streptococcal endocarditis.

The issue of how to confirm penicillin-streptomycin synergism in such isolates is problematic. In our experience, time-kill studies with viridans streptococci are more difficult to perform reliably than are studies with enterococci. It would be expected that, in the absence of "high-level" resistance to the aminoglycoside, synergism would occur. However, appropriate screening concentrations for high-level resistance to aminoglycosides have not been established. For streptomycin, screening concentrations of 2,000 μg/ml as used for enterococci may be too high in light of a recent report documenting lack of in vitro or in vivo synergism against strains of *Streptococcus bovis* with streptomycin MICs of 1,000 μg/ml (56). This observation would suggest that screening tests carried out with streptomycin at 500 μg/ml would be more reassuring in the absence of growth, while strains growing at this concentration could be studied further by time-kill curve techniques or assumed to be resistant to synergism between penicillin and streptomycin. At present, there are insufficient published data to provide an estimate of the frequency of high-level resistance to streptomycin. One recent study, however, indicated that 2% of streptococcal isolates at the Mayo Clinic were highly resistant to this drug. Older data from France suggested resistance in closer to 5% of isolates (85). High-level resistance to gentamicin, if it occurs at all, must be quite rare. While the routine use of penicillin-gentamicin combinations would circumvent any need for testing streptomycin, it is important to note that there is very little published documentation supporting the assumption that this combination is therapeutically equivalent to penicillin-streptomycin, although there is no reason to believe that it is not (182). Also, differences in potential toxicities of the two aminoglycosides must be appreciated (54).

Gram-Negative Bacilli

Value of combination therapy. There is still considerable debate about the role of antibiotic combinations in the treatment of infections due to gram-negative bacilli. On the one hand, several studies provide evidence for comparable results between single-agent and combination therapy. In randomized trials of (i) cefoperazone ± amikacin in cancer patients, some of whom were neutropenic (146), and (ii) aztreonam-vancomycin ± amikacin in neutropenic patients (94), response rates were not significantly better in study arms in which the aminoglycoside was added. In a review of over 400 cases of *Pseudomonas* bacteremia, Bodey et al. (19) noted that, whether or not patients were neutropenic, regimens consisting of an antipseudomonal beta-lactam alone were as effective as those utilizing combinations of such agents with aminoglycosides. Other studies have been unable to provide convincing evidence of any correlation between in vitro synergism and clinical efficacy (36, 143).

On the other hand, support for a role of synergism in the treatment of gram-negative infections comes from several sources. Reyes et al. (150), examining 30 strains of *P. aeruginosa* obtained from patients with bacterial endocarditis, found that synergism (MIC checkerboard; in cation-supplemented Mueller-Hinton broth; using the criterion of a fourfold reduction in the MIC of each antibiotic used in combination) was necessary for, but did not assure, medical cure with carbenicillin combined with gentamicin or tobramycin. Klastersky et al. (100) performed a double-blind trial comparing amikacin-penicillin with amikacin-carbenicillin combinations for treatment of serious gram-negative infections in non-neutropenic patients. Combinations synergistic in vitro against the pathogen being treated were associated

with significantly better outcomes than were seen with nonsynergistic regimens, although the differences were not striking (66 versus 48% favorable outcomes). An almost identical difference in response rates when synergistic or nonsynergistic combinations (determined retrospectively) were used in the treatment of gram-negative rod bacteremias was noted by Anderson et al. (4). Of 173 organisms susceptible to both antibiotics of a given combination, those patients whose organisms were synergistically inhibited (MICs reduced fourfold) responded in 80% of instances, while a 64% response was noted with nonsynergistic combinations. These effects were particularly striking for *P. aeruginosa* infections, in which 10 of 12 responded to synergistic therapy, while none of the 6 responded to nonsynergistic therapy. (The significance of such observations is discussed in detail elsewhere [19].) The presence or absence of synergism was also a significant factor relating to outcome in patients with neutropenia, shock, and "rapidly or ultimately fatal" diseases.

In the treatment of gram-negative bacteremia in neutropenic patients, susceptibility to both components of a combination regimen appears to be beneficial (103). Love et al. (115), for example, noted responses in 75% of patients with pathogens susceptible to both components, while 44% responded if the organism had been susceptible to only one drug. These data are based on a review of patients entered in several clinical trials of various beta-lactam–aminoglycoside combinations. Likewise, in a multicenter trial comparing combinations of amikacin with azlocillin, ticarcillin, or cefotaxime, response of single gram-negative rod bacteremias in neutropenic patients occurred in 66% of patients with strains susceptible to both agents but in only 21% of those whose strains were resistant to the beta-lactam (99). Unfortunately, since no amikacin-resistant beta-lactam-susceptible strains were encountered, and in view of the fairly well-established lack of efficacy of aminoglycosides alone under such circumstances (19), it is impossible to determine whether the difference in outcome was due to any benefit of combination therapy per se or resulted only from the lack of aminoglycoside efficacy. In these studies, the potential role of synergism was not addressed. In a review of patients treated between 1979 and 1982 at their institution, DeJongh et al. (43) found that (MIC checkerboard) synergism had a positive impact on response only among patients with profound (<100 per μl), persistent neutropenia. Of these, 44% treated with synergistic regimens responded versus none of 13 treated with nonsynergistic regimens. Even among patients treated with two antibiotics to which the pathogen was susceptible, synergism was still noted to be an independently beneficial factor. However, confounding the ability to draw firm conclusions about the merits of synergy per se from this study was the fact that, even in the group of persistently profound neutropenic patients, response occurred in 40% of patients treated with regimens containing one component to which the organism was so exquisitely susceptible that synergism could not be assessed.

Such results are at the heart of a major question concerning the role of synergism in gram-negative bacillary infections: that is, if there is any benefit of "synergistic" combinations, does that benefit derive primarily from the enhanced serum bactericidal activity which often results, or are there specific benefits attributable to combination of agents which exert inhibitory or bactericidal effects by different mechanisms (as is the case for penicillin-aminoglycoside combinations against enterococci) (43)? Among patients with gram-negative bacteremia, high SBTs do correlate with favorable clinical response. In nongranulocytopenic patients, SBTs of ≥1:8 correlate strongly with a positive outcome (100, 163). Among granulocytopenic patients, peak SBTs of ≥1:16 appear to be more predictive of a favorable response (>90% in reference 163); among patients with SBTs of <1:8 (163) or <1:16 (44), failure was noted in 83 and 60%, respectively. With several of the newer beta-lactam antibiotics, SBTs against gram-negative bacteria are not only higher but also sustained over a longer period of time than SBTs achievable with older beta-lactam–aminoglycoside combinations (173). These results may explain in part the success of single-drug therapy of gram-negative infections in cancer patients, using agents such as imipenem (18) or ceftazidime (± vancomycin) (106, 148). Although, as discussed earlier, there has been concern about the development of resistant organisms during beta-lactam monotherapy, studies by Aronoff and Shlaes (10) suggest that use of drugs with achievable serum concentrations which vastly exceed inhibitory concentrations is likely to result in a low likelihood that resistant strains will emerge.

In view of the fact that single beta-lactams have been successfully used in patients with serious gram-negative bacillary infections, even in those with neutropenia, it is not surprising that double beta-lactam therapy has met with some success. Combinations such as moxalactam-ticarcillin and moxalactam-piperacillin have shown overall efficacies in neutropenic patients similar to those of comparative beta-lactam–aminoglycoside combinations (94, 185). However, responses in specific patient subgroups such as those with gram-positive coccal (94) or *Pseudomonas* (152, 185) infections are occasionally suboptimal. As more beta-lactam-resistant strains emerge, there is some concern about whether double resistance will be encountered with increasing frequency. In fact, a possible decline in the response of *P. aeruginosa* infections to double beta-lactam therapy during recent years has been discussed (42). While it is difficult to comment on the significance of such reports in light of the numerous variables undoubtedly involved, that response to beta-lactam–aminoglycoside combinations has not declined in parallel suggests that this finding is not artifactually due to methodological changes over time.

A special category of double beta-lactam interactions involves combinations of amdinocillin with a variety of other penicillins or cephalosporins, given the notable PBP affinities of this compound (170). Synergistic effects of such combinations are highly strain specific (137). Furthermore, synergism, as determined by comparison of zone sizes from combination disks and single-drug disks, was not associated with enhanced clinical efficacy (97). There is some evidence that synergism as defined by enhanced killing rates may be more reliable than MIC or MBC synergism criteria in predicting enhanced efficacy in animal models of meningitis (69). Given the rather restricted clinical indications for use of amdinocillin (urinary tract infection or urosepsis) and the availability of numerous alternatives for such therapy, any requirement to test prospective combinations specifically against each pathogen would represent a serious impediment to use of this drug.

Testing of combinations. In vitro tests of synergism against gram-negative bacilli are problematic for several reasons, not the least of which is the uncertain significance of synergism, or lack thereof, in most clinical situations. Furthermore, for gram-negative bacillary infections in critically ill patients (shock, neutropenia, rapidly fatal underlying disease, etc.) for whom synergism is most likely to be beneficial (4), the outcome is often determined long before

results of formal synergy studies can be obtained for the individual patient.

In rare cases of endocarditis due to gram-negative bacilli (other than the fastidious HACEK group), or perhaps for therapy of osteomyelitis or other infections against which long courses of bactericidal antibiotics are desirable, synergy testing in the clinical laboratory can be justified. Prospective regimens for such therapy will usually include a beta-lactam and an aminoglycoside, in an attempt to achieve bactericidal synergism. As a result, time-kill curve methods are preferable. Choice of antibiotic concentrations is purely arbitrary, but use of one drug in subinhibitory concentrations will optimize the likelihood of distinguishing synergism from an additive effect. On the other hand, incorporation of clinically achievable (even if not necessarily subinhibitory) concentrations of both drugs would permit an estimate of the maximum killing rates expected. For penicillins which may be inactivated during prolonged incubation, sampling times at 6 to 7 h rather than 24 h may be preferable (66). An alternative approach, and one which is undoubtedly easier, is to choose therapy based on susceptibility studies and subsequently to use serum bactericidal titer determinations to assess the appropriateness of the selection.

Diffusion techniques are of little value in common practice, except when used in the form of commercially prepared combination disks used to assess activities of trimethoprim-sulfamethoxazole or penicillins with beta-lactamase inhibitors. Disk approximation tests may be used to demonstrate antagonism between two beta-lactams, but results of such studies are unlikely to be the sole source of pivotal information on which to base major therapeutic decisions.

CONCLUSIONS

Antibiotic combinations are commonly used for patient therapy. In the vast majority of cases, such combinations are used to provide a broad spectrum of activity or in the hope of delaying or suppressing the emergence of drug-resistant subpopulations. Tests of antibiotic interactions are neither necessary nor practical in such circumstances. The use of antibiotic combinations in an attempt to produce bactericidal synergism would merit confirmatory testing in many circumstances. However, with enterococci and viridans streptococci, screening for high-level aminoglycoside resistance suffices, in most situations, to predict the presence or absence of synergism when these agents are combined with cell wall-active antibiotics.

Mechanisms of antibiotic interactions against gram-negative bacilli are potentially much more complex. Studies examining the clinical role of synergism against serious infections caused by such organisms yield conflicting results. There may well be an advantage to use of synergistic combinations in patients with prolonged profound neutropenia or in those with endocarditis. Even here, however, newer agents which achieve high serum bactericidal activity as single agents may prove comparable in efficacy to synergistic combinations of older drugs. Selection of in vitro tests for interactions between antibiotics against gram-negative organisms is complicated by lack of standardized techniques, although possible options have been suggested. Fixed-dose antibiotic combinations, such as trimethoprim-sulfamethoxazole and beta-lactam/beta-lactamase–inhibitor combinations, are often useful; testing of these is simplified by the availability of combination disks (microtiter wells, etc.). Fortunately, justifiable requests to test other combinations of agents against gram-negative pathogens are uncommon. Even in these circumstances, alternative tests such as serum bactericidal titers provide useful information which often circumvents the need for formal testing of antimicrobial interactions.

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